JOURNAL OF VIROLOGY, May 2006, p. 4656–4663 Vol. 80, No. 10
0022-538X/06/$08.00+0 doi:10.1128/JVI.80.10.4656–4663.2006

Processing of the Bovine Spongiform Encephalopathy-Specific Prion Protein by Dendritic Cells

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Received 4 January 2006/Accepted 28 February 2006

Dendritic cells (DC) are suspected to be involved in transmissible spongiform encephalopathies, including bovine spongiform encephalopathy (BSE). We detected the disease-specific, protease-resistant prion protein (PrP^{bse}) in splenic DC purified by magnetic cell sorting 45 days after intraperitoneal inoculation of BSE prions in immunocompetent mice. We showed that bone marrow-derived DC (BMDC) from wild-type or PrP-null mice acquired both PrP^{bse} and prion infectivity within 2 h of in vitro culture with a BSE inoculum. BMDC cleared PrP^{bse} within 2 to 3 days of culture, while BMDC infectivity was only 10-fold diminished between days 1 and 6 of culture, suggesting that the infectious unit in BMDC is not removed at the same rate as PrP^{bse} is removed from these cells. Bone marrow-derived plasmacytoid DC and bone marrow-derived macrophages (BMM) also acquired and degraded PrP^{bse} when incubated with a BSE inoculum, with kinetics very similar to those of BMDC. PrP^{bse} capture is probably specific to antigen-presenting cells since no uptake of PrP^{bse} was observed when splenic B or T lymphocytes were incubated with a BSE inoculum in vitro. Lipopolysaccharide activation of BMDC or BMM prior to BSE infection resulted in an accelerated breakdown of PrP^{bse}. Injected by the intraperitoneal route, BMDC were not infectious for alymphoid recombination-activated gene 2^0/common cytokine γ chain-deficient mice, suggesting that these cells are not capable of directly propagating BSE infectivity to nerve endings.

Prion diseases, such as Creutzfeldt-Jakob disease (CJD) in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE) in cattle, are transmissible spongiform encephalopathies that are induced by a novel class of infectious agents thought to be composed of a misfolded, host-encoded, protease-resistant protein, the disease-specific prion protein (PrP^{sc}, PrP^{bse}, or PrP^{cjd} for the isoform of scrapie, BSE, or CJD, respectively). A new form of human disease, called variant CJD (vCJD), emerged as a consequence of transmission from cattle to human due to consumption of BSE-contaminated food. Blood is infectious in sheep infected with the cattle-derived BSE strain, in mice infected with the human-derived vCJD strain, and probably in human vCJD (10, 15, 25, 32). PrP-null (PrP^0) mice are resistant to infection by prions, thus clearly establishing the role of normal, self PrP in prion propagation (6). Immune cells are probably involved in prion pathogenesis, as severely combined immunodeficient (SCID) mice are resistant to peripheral inoculation with a scrapie agent (20). Following a peripheral inoculation, prions have a primary replication phase in the spleen and the lymph nodes (LN) before reaching the brain. Splenic infectiosity is associated with B and T lymphocytes and follicular dendritic cells (FDC) (29, 34). Specific complement components are involved in the initial trapping of prions in lymphoreticular organs early after infection (21). Prion replication does not seem to alter the immune system or to be pathogenic in tissues other than the central nervous system (1). On the other hand, chronic inflammatory conditions may expand the tissue distribution of prions in inflammatory foci, with ectopic induction of PrP-expressing FDC (13).

How prions are transported from the site of peripheral exposure to FDC and to the nervous system is not known. Hematopoietic cells, such as macrophages and dendritic cells (DC), are suspected to be involved in prion neuroinvasion because of their implication in the uptake and transport of antigens from sites of exposure to lymphatic tissue (3). PrP^{sc} was detected in LN resident macrophages in scrapie-infected sheep as well as in scrapie-infected tumor necrosis factor receptor 1-null mice (2, 14, 33). LN from these mice were infectious, suggesting that cells of the immune system, such as macrophages, DC, or lymphocytes, could act as a replication site or as a reservoir for prions. The disease-associated form of PrP was also detected in LN DC from scrapie-infected rats, as well as in cerebral blood vessel DC in human sporadic CJD and vCJD (16, 22). $CD205^+$ DC were detected in several regions of the brain, such as the cerebral cortex and thalamus, in mice intraperitoneally infected with the mouse-adapted KFu strain of Gerstmann-Straussler-Scheinker syndrome (36). DC purified from spleens of scrapie-infected mice were infectious

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for immunodeficient mice following peripheral inoculation, but only when live DC were injected (4).

We focused in the present study on the role of DC in BSE physiopathology. As DC are a heterogeneous population of cells, including myeloid, lymphoid, and plasmacytoid subsets, our objective was to determine which DC populations express PrP and are responsible for transmitting the disease. Following combined intraperitoneal and intracerebral injections with BSE cow brain homogenate, only 4/22 SCID mice developed prion disease (5). In contrast, 100% of normal competent mice died of BSE. These findings indicate that the immune system probably plays an important role in the cow-mouse species barrier. To solve the species barrier problem, we used the BSE mouse-adapted prion strain 6PB1 in challenges with immunodeficient mice (23). We first investigated the uptake of PrP^{bse} by spleen DC in vivo following a peripheral infection by BSE prions. As spleen DC cannot be maintained in culture for more than a few days, we chose the bone marrow-derived DC (BMDC) model to investigate the uptake of PrP^{bse} by DC in vitro. We analyzed both prion infectivity and PrP^{bse} processing in cultures of BMDC incubated with BSE prions. Then, we compared this processing to those of bone marrow-derived plasmacytoid DC (BMpDC), bone marrow-derived macrophages (BMM), and T and B lymphocytes isolated from spleen by magnetic antibody cell sorting.

MATERIALS AND METHODS

Animals. Six- to 8-week-old C57BL/6J (Janvier, Le Genest-Saint-Isle, France), PrP^0 (CDTA, France), B6129SF1/J (the F1 cross between C57BL/6J and 129S, stock number 101043; Jackson ImmunoResearch Laboratories), and recombination-activated gene 2^0/common cytokine γ chain-deficient (RAG γc^0) immunodeficient mice were used for this study (7, 11, 12). RAG γc^0 mice originated on a 129SF1 background were backcrossed on the C57BL/6 background. All animals were housed in level 3 care facilities of the Pasteur Institute, officially registered for prion experimental studies with rodents (Ministry agreement number A 75-15-27 for animal care facilities and agreement number 75-585 for animal experimentation). RAG γc^0 immunodeficient mice were kept in isolators under specific-pathogen-free conditions.

Infectious material. The mouse-adapted BSE strain 6PB1 was kindly provided by D. Dormont (CEA, Fontenay-aux-Roses, France). It was stabilized and propagated in C57BL/6 mice (24). The inoculum was a brain homogenate at 10% (wt/vol) in 5% glucose solution from BSE-infected mice at the terminal stage of disease, routinely titrating 5×10^8 to 5×10^9 mean 50% lethal doses (LD_{50}) per gram in intracerebral-infection challenges. The mean (±standard deviation) survival time was around 181 ± 5.4 days after intracerebral infection or 315 ± 27.3 days after intraperitoneal challenge (23). Healthy mouse brain homogenate was used as a negative control.

SAF isolation. Scrapie-associated fibrils (SAF) were purified from infected brains according to the method developed by Vilette et al. (38), which was slightly modified (38). Briefly, 1 volume of 20% NaCl (Sigma, St. Louis, MO) was added to 1 volume of 20% brain homogenate (BSE or control). After homogenization and addition of 1 volume of a mix containing 20% Sarkosyl (Interchim, Montluçon, France), 2% SB3-14 (Calbiochem, VWR, Fontenay-sous-Bois, France), and 2 mM Tris-HCl (pH 7.4), samples were incubated for 15 min at room temperature. They were then centrifuged at 26,500 × *g* for 2 h at 20°C on a 20% sucrose cushion. Pelleted material was resuspended in Tris-borate-sodium buffer and heated at 80°C for 10 min before use for cell infections.

Preparation of spleen DC. DC were purified by magnetic cell sorting as previously described (30). Briefly, spleens were removed from mice and treated for 45 min at 37°C with 400 U/ml of collagenase type IV and 50 μg/ml of DNase I (Roche, Mannheim, Germany) in RPMI 1640. After inhibition of collagenase activity with 6 mM EDTA in phosphate-buffered saline (PBS), spleens were dissociated in $Ca2^+$- and $Mg2^+$-free PBS in the presence of 2.5 mM EDTA, 0.5% fetal calf serum (Invitrogen, Cergy-Pontoise, France), and 1% antibiotics (penicillin, 100 U/ml; streptomycin, 100 μg/ml). Single spleen cell suspensions were prepared and incubated with colloidal superparamagnetic microbeads conjugated to anti-CD11c monoclonal antibody (MAb) (MACS–anti-CD11c, N418 clone; Miltenyi Biotec, Bergisch-Gladbach, Germany) by following the manufacturer's instructions. $CD11c^+$ cells were positively selected by two serial passages with miniMACS magnetic cell sorting (Miltenyi Biotec). The purity of DC preparations was superior to 70% after the first column and superior to 93% after the second one.

Preparation of T and B spleen cells. T and B cells were purified from mouse spleen by magnetic cell sorting with pan-T and -B cell isolation kits, respectively, following the manufacturer's instructions (Miltenyi Biotec). The purity of both T and B populations was superior to 97%.

Culture medium. Complete medium consisted of RPMI 1640 containing L-alanyl-L-glutamine dipeptide (Invitrogen, Cergy-Pontoise, France) supplemented with 10% fetal calf serum (ICN Pharmaceuticals France S.A., Orsay, France), 50 μM 2-mercaptoethanol, and antibiotics (penicillin, 100 U/ml; streptomycin, 100 μg/ml).

Generation of BMM, BMDC, and BMpDC. BMM, BMDC, and BMpDC were generated from bone marrow precursors, as previously described (17). Briefly, bone marrow cells from C57BL/6 or PrP-null mice were harvested and plated at 2×10^5 cells/ml in 60-mm cell culture dishes (BD Falcon, Bedford, Mass.) in complete medium with 5% of a supernatant containing granulocyte-macrophage colony-stimulating factor (GM-CSF), 5% of a supernatant containing fms-like tyrosine kinase 3 ligand (Flt3-L), or 10% of a supernatant with macrophage colony-stimulating factor for the generation of BMDC, BMpDC, or BMM, respectively. J558 GM-CSF (a kind gift from T. Rolink, Basel, Switzerland), Sp210 Flt3-L (a kind gift from R. Rottapel, Toronto, Canada), and L929 (ATCC, Teddington, Middlesex, United Kingdom) cell lines were used to produce GM-CSF, Flt3-L, and macrophage colony-stimulating factor, respectively. BMpDC were purified prior to infection by magnetic cell sorting using a murine plasmacytoid dendritic cell antigen 1 isolation kit following the manufacturer's instructions (Miltenyi Biotec). The purity of BMpDC preparations was superior to 86%.

Prion infection of cultured cells. T and B splenic cells were infected just after purification by magnetic cell sorting. Infections of BMDC and BMM were performed on day 6. Infection of BMpDC was performed on day 6, after purification by magnetic cell sorting. For in vitro activation, BMM or BMDC were incubated at day 5 for 24 h with 10 μg/ml of lipopolysaccharide (LPS) (Invivogen, San Diego, Calif.) prior to infection. The murine SAF from 1 mg of brain homogenate of uninfected or BSE terminal-stage mice or 1 mg of direct brain homogenate was incubated with 4×10^6 cells in 60-mm cell culture dishes (BD Falcon, Bedford, Mass.). Inocula were previously heated at 80°C for 10 min to avoid bacterial contamination. The nonadherent and semiadherent cells were recovered by flushing the plates with PBS containing 5 mM EDTA and washed in PBS before fluorescence-activated cell sorter (FACS) and Western blot analysis or before injection into wild-type or RAG γc^0 mice.

Western blot analysis. Briefly, tissue homogenates (5% [wt/vol] in 5% glucose) or cell lysates (8×10^5 cells in 0.5% sodium deoxycholate [Sigma] and 0.5% NP-40 [Coger, France]) were treated or not for 1 h at 37°C with 10 or 40 μg/ml of proteinase K (PK) (Sigma, France), respectively. Laemmli buffer (Bio-Rad, Ivry sur Seine, France) containing 3 M (final concentration) urea was added, and 20 μl of each extract, corresponding to 100 μg of brain or to 1.6×10^5 cultured cells, was used for PrP^{bse} detection. Samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 12% polyacrylamide gels and electroblotted onto nitrocellulose membranes. Immunodetection was done by using a pool at 1 μg/ml of two or three mouse monoclonal anti-PrP antibodies, all raised against hamster PrP (SAF32, recognizing the octo-repeat region located in the N-terminal part of PrP; SAF84, recognizing an epitope located between 160 to 170 residues; and SAF83, with an unknown recognized epitope [all from Spi-Bio, Massy, France]), followed by a peroxidase-conjugated goat anti-mouse antibody (Jackson ImmunoResearch Laboratories). Immunoreactivity was visualized by enhanced chemiluminescence (ECL kit; Pierce, France).

PrP immunostaining of DC. Immunocytochemistry was run on fixed, frozen DC as previously described, with minor modifications (37). Cells were fixed for 15 min in 4% formaldehyde in PBS, incubated in cold acetone for 10 min, and cryoprotected in 15% sucrose for 15 min before freezing. Two combined monoclonal anti-PrP antibodies were used: SAF84 (1 μg/ml) and SAF83 (1 μg/ml) from SpiBio. SAF84 gave a weaker immunostaining than SAF83. Slides were treated with 5 M guanidine for 5 min before PrP staining. Staining was performed with a mouse ABC staining system (Santa Cruz Biotechnology). Images were taken with a Leica DMLBHC microscope by using a Leica DC 100 charge-coupled-device camera and Leica IM50 image manager software (Leica Microsystems, Rueil-Malmaison, France).

Flow cytometry analysis. Cells were washed and resuspended in PBS containing 1% bovine serum albumin and 0.2% sodium azide (both purchased from Sigma), incubated with anti-CD16/CD32 Fc Block (2.4G2 clone; BD Pharmin-

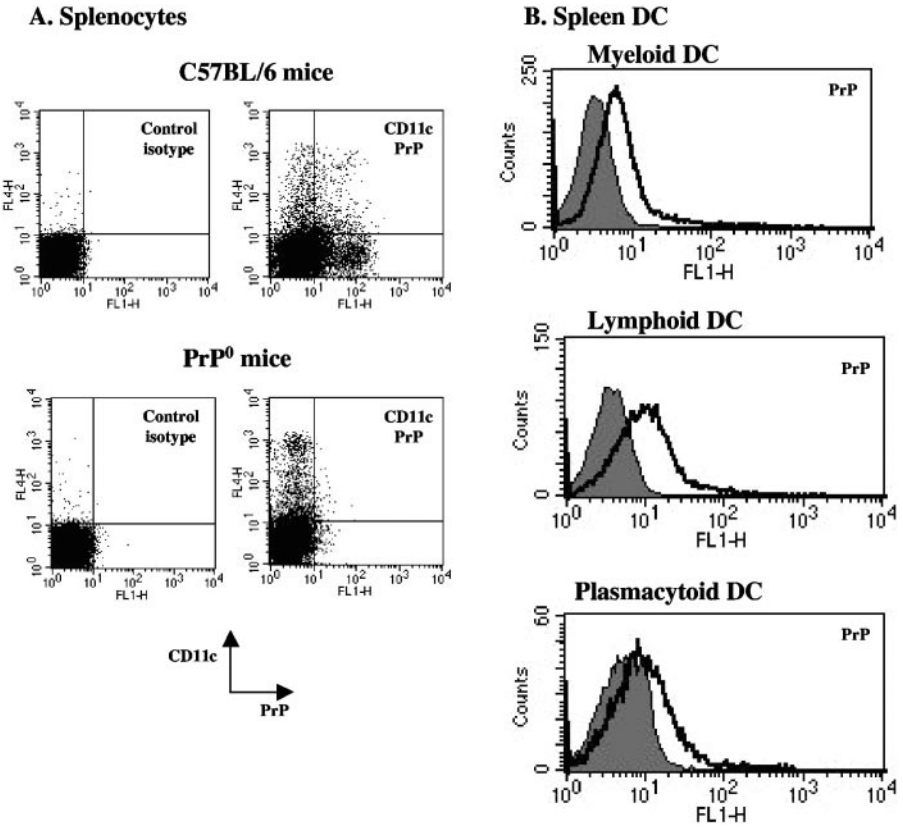

FIG. 1. Expression of PrP on spleen DC. Spleen DC of C57BL/6 or PrP^0 mice were analyzed by flow cytometry for the expression of CD11c, CD11b, B220, and PrP. (A) Dot plot analysis of CD11c versus PrP labeling. No PrP was detected in PrP^0 DC. (B) Expression of PrP on myeloid DC ($CD11c^+$ $CD11b^+$), lymphoid DC ($CD11c^+$ $CD11b^-$), and plasmacytoid DC ($CD11c^+$ $B220^+$) is shown by histograms. Gray shading indicates appropriate isotype control. The results are representative of three experiments.

gen, San Diego, Calif.) to avoid antibody binding via Fc, and labeled at 4°C for 20 min with different combinations of the following fluorescein isothiocyanate-, phycoerythrin-, or allophycocyanin-conjugated MAbs: anti-B220 (RA3-6B2), anti-CD11c (HL-3), anti-CD11b (M1/70), anti-CD3 (17A2), polyclonal goat anti-mouse immunoglobulin (Ig), and appropriate Ig isotype controls. All MAbs were purchased from BD Pharmingen, except anti-PrP (SAF83; SpiBio, France). SAF83 was conjugated to fluorescein isothiocyanate (Sigma) or to Alexa 488 by use of a Zenon mouse IgG labeling kit (Molecular Probes, PoortGebouw, The Netherlands) prior to staining. Cells were analyzed with CellQuestPro software (BD Biosciences) on a FACSCalibur flow cytometer (BD Biosciences, San Diego, Calif.) situated in our level 3 care facilities.

BSE diagnosis. Animals were examined three times per week for clinical signs of BSE, which could include bradykinesia, waddling gait, poor coat condition, terminal incoordination, and weight loss. BSE-infected mice, animals affected by injuries, and surviving mice were euthanized by cervical dislocation at the terminal stage of the disease or at 650 days postinoculation by following the Pasteur Institute's animal experimentation regulations. BSE diagnosis was confirmed by detection of PrP^{bse} by Western blotting after PK digestion. The survival period was calculated as the interval between the first inoculation and the death, or in extremis, stage. The BSE incidence and the mean survival time were determined for each group of mice. Statistics were performed by use of Prism software (GraphPad Software, San Diego, Calif.).

Infectivity measurements. End point titration was performed by intracerebral inoculations of serial decimal dilutions of the infectious inoculum, ranging from 10^{-2} to 10^{-10}. Twenty microliters of inoculum was injected into groups of four to six C57BL/6 mice. The infectious titer was determined by the calculation of the LD_{50} according to the formula of Reed and Muench (35).

Adoptive cell transfer. At day 1 or 6 postinfection, wild-type or PrP-null BMDC semiadherent cells were washed and suspended in PBS. Live BMDC (4×10^6) in 500 μl PBS were immediately injected by the intraperitoneal route into groups of four to six RAG γc^0 mice. In parallel, as a control, four to six RAG γc^0

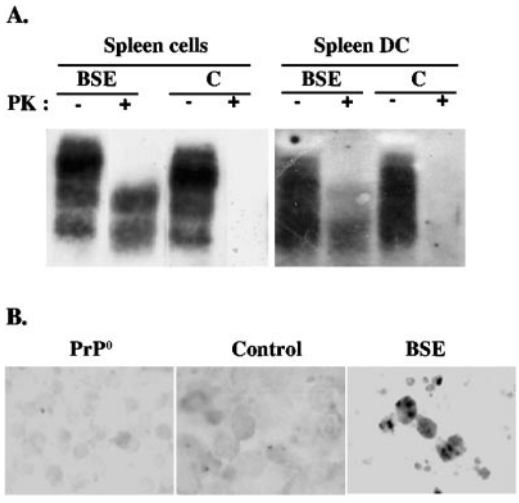

FIG. 2. PrP^{bse} is expressed in spleen DC of BSE-infected mice at 45 days postinjection. (A) Immunoblots show the accumulation of PK-resistant PrP^{bse} in total spleen cells and purified $CD11c^+$ DC from C57BL/6 mice intraperitoneally inoculated with BSE and analyzed at day 45 postinfection. The treatment of samples in the presence (+) or the absence (−) of PK is indicated. C, control (C57BL/6 noninfected mice). (B) PrP immunostaining of $CD11c^+$ DC purified from PrP^0, C57BL/6 noninfected (Control), and BSE-infected (45 days post-intraperitoneal infection) mice. Magnification, ×40.

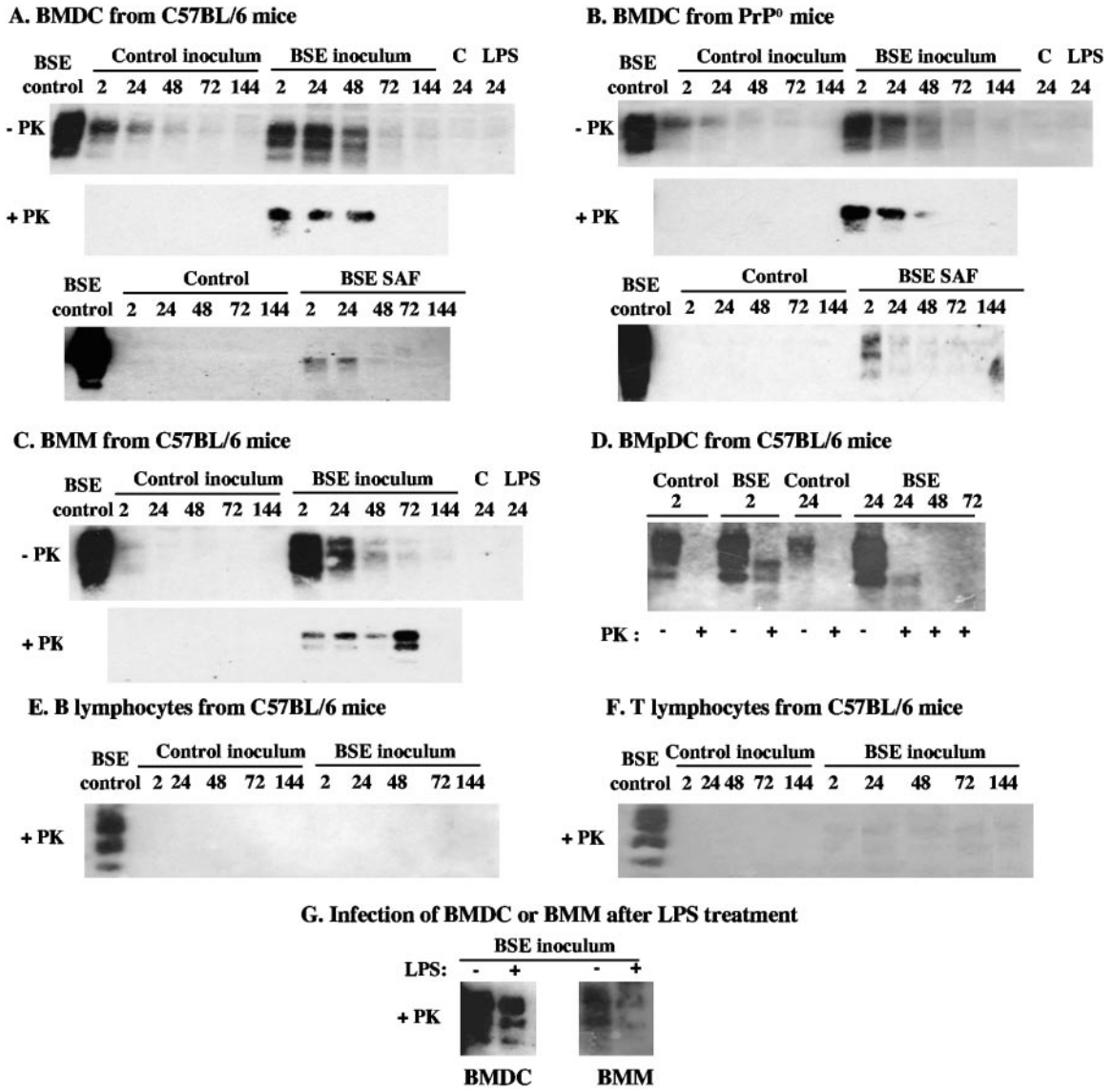

FIG. 3. Uptake and degradation of PrP^{bse} by BMM, BMDC, and BMpDC. Immunoblots show levels of PrP in BMDC (A and B), BMM (C), BMpDC (D), and splenic B (E) and T (F) lymphocytes untreated or treated with LPS, SAF, control, or BSE inoculum. Panel G shows PrP^{bse} levels in LPS-activated BMDC or BMM following 2 h of exposure to the BSE inoculum, a brain homogenate from noninfected or terminally BSE-sick mice. SAF were extracted from control or BSE brain homogenate. The period of treatment (2, 24, 48, 72, or 144 h) is indicated. BMDC, BMpDC, and BMM were prepared from C57BL/6 (A, C, and D) or PrP^0 (B) mice. Control samples included untreated BMDC (labeled C), BMDC treated with SAF from control homogenate (labeled Control), and BMDC treated with SAF from 20 μg undigested terminally BSE-infected brain (labeled BSE SAF). Samples were digested (+) or not (−) with 40 μg/ml PK. Immunoblots with undigested or PK-digested samples were revealed with anti-PrP antibodies SAF32 and SAF84.

mice were injected by the same route with the same amount of BMDC that had been killed by three cycles of freezing and thawing.

RESULTS

Detection of PrP^{bse} in spleen DC of BSE-infected mice. To unravel the role of DC in BSE pathology, we investigated the expression of both normal and misfolded PrP isoforms in spleen DC of mice inoculated or not with BSE prions. For noninfected C57BL/6 mice, FACS analysis showed that splenic $CD11c^+$ DC express membrane PrP (Fig. 1A). A high expression of PrP was noted in 35% of splenic $CD11c^+$ DC. As expected, no PrP expression was detected in PrP^0 mice (Fig. 1A). To determine which subsets of splenic DC express PrP, spleen DC were analyzed by flow cytometry for the expression of CD11c, CD11b, B220, and PrP. As shown in Fig. 1B, myeloid ($CD11c^+$ $CD11b^+$), lymphoid ($CD11c^+$ $CD11b^-$), and plasmacytoid ($CD11c^+$ $B220^+$) DC express PrP on their surfaces. The highest expression of PrP was detected in lymphoid DC and the lowest in plasmacytoid DC. Similarly, we found that cultured BMDC, BMpDC, and BMM express PrP on their surfaces (data not shown).

In BSE-infected C57BL/6 mice, at 45 days after intraperitoneal inoculation, PrP^{bse} in total spleen cells was detectable by Western blotting as well as in $CD11c^+$ DC purified by mag-

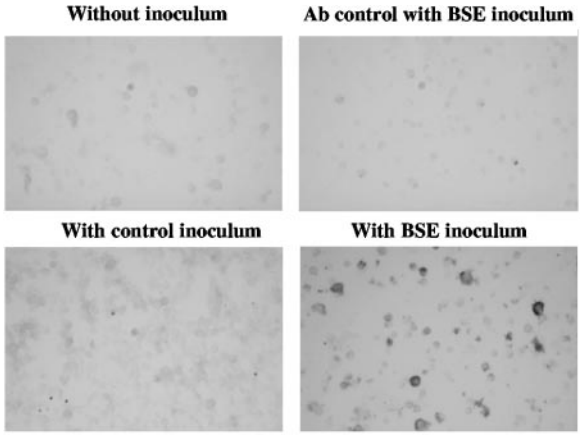

FIG. 4. Detection of PrP^{bse} in BMDC of PrP-null mice after in vitro infection with BSE prions. Immunocytochemical analysis detected the presence of PrP in BMDC treated with BSE inoculum for 24 h (lower right panel) but not in cells exposed to control uninfected inoculum (lower left panel). A control untreated BMDC is shown in the upper left panel. Antibody (Ab) control staining of BSE inoculum-treated BMDC is shown in the upper right panel. Magnification, ×40.

netic cell sorting (Fig. 2A). DC from noninfected control mice were negative for PrP^{bse}. These data were confirmed by PrP immunostaining of splenic DC purified by magnetic cell sorting (Fig. 2B). A strong intracellular labeling of PrP was observed with DC purified from spleens of mice with preclinical BSE but not with those from PrP^0 or noninfected C57BL/6 mice. Altogether, these data strongly suggest that murine splenic DC express normal PrP and PrP^{bse}, and thus these cells could be infected in vivo in the BSE preclinical stage.

In vitro capture and processing of PrP^{bse} by BMM, BMDC, and BMpDC. Cell cultures were exposed at day 6 to BSE brain homogenate or to PrP^{bse} purified in SAF. The control inoculum was LPS, brain homogenate, or SAF from healthy mice.

Western blot analysis showed that PrP^{bse} of the BSE inoculum was taken up within 2 h by BMDC (Fig. 3A). PrP^{bse} was then cleared in 2 to 3 days. Then, long-term cultures showed no PrP^{bse} expression, strongly suggesting that BMDC did not multiply BSE prions in vitro (data not shown). BMDC from PrP^0 mice also acquired and cleared PrP^{bse} in vitro with a similar time course (Fig. 3B). Similarly, SAF were taken up and cleared in vitro (Fig. 3A and B). Normal PrP of the control inoculum was processed and rapidly degraded by BMDC from normal or PrP^0 mice (Fig. 3A and B). Similarly, BMM and BMpDC acquired and rapidly cleared PrP of the control or BSE inoculum (Fig. 3C and D). LPS activation of BMDC or BMM before BSE infection resulted in an accelerated breakdown of PrP^{bse} (Fig. 3G). Splenic T or B cells acquired only small amounts of PrP^{bse} and did not multiply it (Fig. 3E and F). To verify that PrP^{bse} was processed by BMDC, PrP immunocytochemistry was run on BMDC of PrP^0 mice after their exposure to the BSE inoculum (Fig. 4). A strong intracellular labeling of PrP in PrP^0 BMDC was detected after 24 h of exposure to the BSE inoculum (Fig. 4). As no staining was detected in BMDC treated or not with control inoculum, these data indicate that only PrP^{bse}, but not normal PrP, was detected in BSE inoculum-treated BMDC, confirming the Western blot analysis results. Furthermore, these immunocytochemistry data also indicate that PrP^{bse} was internalized by BMDC before clearing. Altogether, these findings highlight the specific high capacity of DC and macrophages to capture and clear PrP^{bse} in vitro.

Prion infectivity in BMDC incubated with BSE prions. To quantify prion infectivity in DC cultures, a time course of in vitro infection was performed by in vivo titration of cell lysates (Table 1). After 1 or 6 days of BMDC culture with the BSE inoculum, adherent cells were washed twice before being scraped in PBS, frozen, and injected without filtration into the brains of C57BL/6 mice. We found that following 1 or 6 days of in vitro incubation with BSE brain homogenate, PrP-expressing or PrP^0 BMDC were highly infectious in vivo. Following 1 day of BSE prion incubation, BMDC from C57BL/6 or PrP^0 mice have an infectious titer of LD_{50} of $10^{4.7}$ and $10^{3.9}$ infectious units per 4×10^6 injected cells, respectively. BMDC were 10-fold less infectious after 6 days of culture than after 1 day.

Fate of RAG γc^0 mice after intraperitoneal injection with BSE-infected BMDC. To further investigate whether DC have the ability to propagate prion infectivity to the central nervous system, we tested, in RAG γc^0 immunodeficient mice, the infectivity of BMDC exposed in vitro to BSE prions. These mice are resistant to murine BSE inoculum (brain homogenate) by the intraperitoneal route, although they are susceptible to the same inoculum after intracerebral inoculation (Table 2). While infected immunocompetent mice accumulated PrP^{bse} in spleen and brain, RAG γc^0 mice intracerebrally infected with BSE brain homogenate accumulated PrP^{bse} in brain but not in spleen (data not shown). These findings confirm that RAG γc^0 immunodeficient mice lack splenic target cells supporting BSE prion multiplication.

Four million live or dead BMDC from normal or PrP^0 mice were incubated for 1 or 6 days with BSE prions and then intraperitoneally injected into RAG γc^0 mice. At 6 months after injection, FACS analysis of blood showed that these mice still lacked B220 cells (data not shown). Seven hundred days after injection, no animal had developed BSE (Table 3), suggesting that BMDC did not directly release infection to nerve terminals.

TABLE 1. Prion infectivity in BMDC incubated with BSE prions: survival times after intracerebral passage into C57BL/6 mice

Parameter	Measure of infectivity (survival time[s] [days] or LD_{50})			
	C57BL/6 BMDC incubated with BSE prions for:		PrP^0 BMDC incubated with BSE prions for:	
	1 day	6 days	1 day	6 days
Dilution of cellular lysate				
0	221 ± 25	276 ± 36	238 ± 10	293 ± 45
10^{-1}	231 ± 15	405 ± 99	228 ± 5	281, 345
10^{-2}	260 ± 31	308, 322	265, 267, 267	390
10^{-3}	245, 307, 400		383	
10^{-4}				
10^{-5}				
LD_{50}/4 M BMDC	$10^{4.7}$	$10^{3.4}$	$10^{3.9}$	$10^{2.6}$

TABLE 2. Susceptibility of RAG γc^0 mice to BSE prions

Deficiency	Mouse strain	Inoculum dilution	Intracerebral route of infection		Intraperitoneal route of infection	
			No. of mice infected/total no. of mice	Survival time (days)[a]	No. of mice infected/total no. of mice	Survival time(s) (days)[a]
T, B, and NK cells	RAG γc^0	10^{-2}	11/12	242 ± 16*	1/12	387
		10^{-4}	ND[b]		0/4	
		10^{-6}	ND		0/4	
PrP	PrP^0	10^{-2}	0/4		0/4	
None	C57BL/6	10^{-2}	13/13	193 ± 4	16/16	333 ± 26
		10^{-4}	ND		4/4	359 ± 35
		10^{-6}	1/4	397	2/5	347, 368
		10^{-7}	0/4		0/4	
		10^{-8}	0/4		0/4	
		10^{-9}	0/4		0/4	
None	B6129SF1/J	10^{-2}	10/10	187 ± 8	ND	ND

[a] Means ± standard deviations are given. *, result is significant versus that for C57BL/6 or B6129SF1/J mice ($P < 0.01$, Mann-Whitney test).
[b] ND, not done.

DISCUSSION

The presented study shows that BMDC, BMpDC, and BMM efficiently capture and remove PrP^{bse} in vitro. In contrast, no uptake of PrP^{bse} was observed when splenic B and T lymphocytes were incubated with a BSE inoculum. Transferred BSE-infected BMDC did not spread BSE in RAG γc^0 mice, suggesting that these cells do not have the ability to directly release infectivity to nerve terminals.

We showed here that myeloid, lymphoid, and plasmacytoid DC purified from spleen express normal PrP at levels conceivably high enough to support replication of PrP^{bse} in vivo. We also found that cultured BMpDC and BMDC, which were mainly myeloid ($CD11b^+$) DC, express large amounts of normal PrP on their surfaces. These data confirm and extend an earlier study showing PrP expression on myeloid DC in humans (8). DC are probably involved in PrP^{bse} uptake in vivo, as we detected PrP^{bse} in spleen DC shortly after intraperitoneal inoculation with BSE strain. Our findings are consistent with those of Huang et al., who also reported the presence of PrP^{sc} in DC shortly after oral infection of rats with ME7 scrapie agent (16). Our data also fit with the demonstration of the infectivity of spleen DC from preclinical scrapie-infected mice (4). DC might be chemoattracted by PrP^{bse}, as a neurotoxic PrP fragment, which can form insoluble amyloid fibrils similar to SAF, is chemotactic in vitro for immature DC (18, 19).

We demonstrated that cultured BMDC and BMpDC efficiently captured and cleared PrP^{bse} within 2 to 3 days of incubation with a BSE inoculum. Our findings with the BSE strain confirm and extend earlier studies with scrapie strains, showing a PrP^{sc} clearance in vitro by rodent BMDC or Langerhans cells (16, 26, 27). As macrophages and DC share several properties, including sample of antigens and motility, we compared the processing of PrP^{bse} in BSE-infected cultured BMM and BMDC. We found that BMM rapidly captured and cleared PrP^{bse} from the BSE inoculum in vitro, with a time course similar to that of BMDC. Our results are in agreement with two reports on in vitro and in vivo clearance of the scrapie agent by macrophages (9). The ability of BMDC and macrophages to capture and degrade PrP^{bse} seems to be specific to these antigen-presenting cells, as we showed here that splenic T and B cells were not able to internalize and breakdown PrP^{bse}. Moreover, we found that LPS-activated BMDC or BMM showed an accelerated breakdown of PrP^{bse}, suggesting that the process of capture and clearance of PrP^{bse} can be increased following DC maturation or macrophage activation. Our results differ from those of Mohan et al., who showed an inhibition of PrP^{sc} clearance when skin-derived DC were activated by LPS (28). This difference could be due to the prion strain or the subset of DC. The kinetics of PrP^{bse} degradation by C57BL/6 and PrP^0 BMDC were very similar, suggesting that the normal PrP expressed by BMDC did not play a role in the capture of PrP^{bse}.

We observed, by in vivo titration, that BMDC degrade BSE prions following in vitro exposure. Our data are consistent with

TABLE 3. Fate of RAG γc^0 mice after intraperitoneal injection with wild-type or PrP-null BMDC infected in vitro by BSE prions

Origin of BMDC	Status of BMDC injected	Day post-in vitro infection	No. of injected RAG γc^0 mice	Survival times (days) after injection	BSE diagnosis by Western blot analysis
C57BL/6	Live	1	6	124, 194, 359, 359, 445, 476	Negative
	Dead	1	4	424, 441, 490, 512	Negative
	Live	6	5	1, 381, 384, 386, 423	Negative
PrP^0	Live	1	4	509, 545, 692, 700	Negative
	Dead	1	4	352, 700, 700, 700	Negative
	Live	6	5	469, 489, 522, 572, 703	Negative

those of Mohan et al., who reported a clearance of the scrapie agent by skin-derived DC following in vitro incubation (28). We found that although PrP^{bse} was not detected within the cell culture at 6 days following incubation with BSE prions, infectivity still exists, confirming that the bioassay presents a very superior sensitivity level of infectivity. Interestingly, we observed only a 10-fold diminution of the infectious titer between days 1 and 6 following incubation with BSE inoculum, suggesting that the infectious unit in BMDC is not removed at the same rate as PrP^{bse} is removed from these cells. PrP^{bse} could probably be cleaved in small infectious peptides, which cannot be detected easily by Western blotting. The minimum PrP peptide size required to propagate infectivity is not yet known. As the infectious titer of C57BL/6 BMDC is 10-fold higher than that of PrP^0 BMDC, these data do not exclude the possibility of some initial amplification of BSE prions in C57BL/6 BMDC versus PrP^0 BMDC.

Immunodeficient RAG γc^0 mice intraperitoneally challenged with BSE-infected BMDC did not develop BSE. These data suggest that BMDC do not release infection to nerve terminals and thus that these cells do not play a role in direct neuroinvasion. These results are in agreement with several earlier observations. First, SCID and RAG immunodeficient mice which possess DC are resistant to peripheral prion infection, indicating that DC are probably not target cells for prions. Second, a treatment depleting DC did not affect scrapie infectivity (31). Third, the neuroinvasion by scrapie following inoculation via the skin was not impaired in CD40 ligand-deficient mice with blocked Langerhans cells, suggesting that this subset of DC is not involved in scrapie transport to draining lymphoid tissue (27). Altogether, these findings do not support the hypothesis that BMDC play a role in direct prion neuroinvasion. Our results differ from an experiment of Aucouturier et al., where the infectivity of spleen DC could be transferred, by a peripheral route, to RAG-deficient mice (4). This discrepancy might be due to the presence of FDC copurified with spleen DC or to the level of infectivity isolated from the in vivo versus the in vitro model. On the other hand, our results do not definitively exclude a role of BMDC in indirect neuroinvasion by prions. Indeed, one can hypothesize that neuroinvasion could occur if the BSE-infected cultured BMDC were allowed to interact with FDC or other immune cells of which RAG γc^0 mice are devoid. Our in vitro data clearly show that BMDC capture PrP^{bse} and internalize it. One can suppose that, like for other antigens, BMDC cleave PrP^{bse} into peptides and express it in the cell surface for interaction/activation of immune cells such as T lymphocytes. If these cells are absent, as in the case of RAG γc^0 mice or in BMDC cultures, the fate of those peptides is probably further reinternalization, digestion, and thus clearance of prion infectivity. If immune cells and FDC are present, one cannot exclude the possibility that BSE-infected BMDC might transfer infectivity to these cells and then to the nervous system.

ACKNOWLEDGMENTS

We thank Monique Dubois-Dalcq, Michel Goldberg, and Pierre-Marie Lledo for expert advice and critical reading of the manuscript, Karim Sebastien and Eddie Maranghi for animal care, Marie-Jésus Rojas for expert technical advice regarding cell cultures, and Jacques Christophe Cossec for the initial Western blot studies.

This work was supported by Bio-Rad, the Pasteur Institute, Paris (PTR 105), France, and GIS 2004 infections à prions. C.R.-B. was supported by a postdoctoral PTR fellowship from the Pasteur Institute.

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